ORIGINAL ARTICLE

Measuring progress to healing: A challenge and an opportunity

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Abstract

Complete healing is problematic as an endpoint for evaluating interventions for wound healing. The great heterogeneity of wounds makes it difficult to match groups, and this is only possible with multivariate stratification and/or very large numbers of subjects. The substantial time taken for wounds to heal necessitates a very lengthy study. Consequently, high quality randomised controlled trials demonstrating an effect of an intervention to a satisfactory level of statistical significance and with a satisfactory level of generalisability are extremely rare. This study determines that the healing of venous leg ulcers receiving multi-component compression bandaging follows a linear trajectory over a 4-week period, as measured by gross area healed, percentage area healed, and advance of the wound margin. The linear trajectories of these surrogates make it possible to identify an acceleration in healing resulting from an intervention, and allows self-controlled or crossover designs with attendant advantages of statistical power and speed. Of the metrics investigated, wound margin advance was the most linear, and was also independent of initial ulcer size.

KEYWORDS
intermediate measure, surrogate, venous leg ulcer, wound margin advance

Key Messages
- randomised controlled trials using complete healing as an endpoint to evaluate interventions for wound healing are frequently under-powered
- statistical sensitivity can be improved by using a short-term surrogate or intermediate endpoint for healing, allowing self-controlled or crossover designs
- wound margin advance is close to linear over a 4-week term, and so is a suitable intermediate endpoint for identifying any changes in healing rate due to an intervention

1 | INTRODUCTION

A great deal of wound-healing research is conducted for the purpose of evaluating products, processes, and pathways that can accelerate wound healing.[1] To that end, an enduring problem is to find an outcome endpoint that can be used robustly. Investigators have not arrived at consensus as to appropriate endpoints to demonstrate that the agent under evaluation actually promotes wound healing.[2] To be useful, endpoints need to be not only accurate, but reproducible and meaningful.

One of several orthodoxies propounded is that the only truly externally valid metric is the number of wounds completely healed in a cohort. A major problem with using complete healing (100% closure) as an outcome for therapeutic efficacy of chronic wounds is that complete healing is frequently only observed after a protracted period. [3]This, combined with the inherent heterogeneity in wounds, means that a conventional randomised controlled trial (RCT) for wound healing would be expected to require hundreds of subjects and take several years. This makes the study so costly and lengthy an undertaking as to render it largely impracticable.[4] It has long been suggested that studies could be effectively be shortened by using short-term healing rate as an outcome.[5]

Additionally, it has been stated[6] that healing time curves (wound-healing trajectories) are a "moving picture" of healing that provide more detail than a "snapshot approach" in which only the proportion of patients healed (100% closed) at the end of the study is assessed. Consequently, there has been a call for surrogate endpoints to be used in wound healing studies.[7]

The surrogate approach consists of measuring the wound at several time intervals to establish its healing rate. Then, after the introduction of the intervention being investigated, the healing rate is measured again to observe any effect. This approach raises further questions as to which metric is best used to quantify the size of the wound, and thus its healing rate. The area of the wound may be measured simply by tracing or by more sophisticated photographic methods.[8] Percentage change in area over 4 weeks has been shown to be a good predictor of ultimate healing in venous leg ulcers (VLUs).[9,10] However, changes in raw area have been shown to exaggerate the importance of large wounds in clinical data, whereas changes in area as a percentage of initial area correspondingly exaggerate small wounds.[11]

Gorin[11] made the observation that, in a cohort of wounds, different wounds will be deemed to be healing faster depending on which metric is used to measure healing. Figure 1 illustrates this point diagrammatically. Consider two wounds (idealised as circular for illustrative purposes): A and B. At initial presentation, wound A has

a radius of 5 cm, and over the course of 4 weeks this reduces to 4 cm. Wound B reduces from a radius of 3–2 cm over the same period.

If we adopt gross area reduction (GAR) as our metric, the results may be seen in Table 1, where we conclude that wound A is healing faster than wound B. However, if we adopt percentage area reduction (PAR) as our metric, Table 2 shows us that wound B is healing faster than wound A. Finally, if we adopt advance of the wound margin in cm as our metric (represented here by a change in the radius of the idealised circular wound), Table 3 gives equal healing rates for wounds A and B.

This apparent anomaly is explained by the quadratic relationship between radius and area (area is proportional to the radius squared) which causes changes in area to be proportionally exaggerated in larger wounds, for a given change in radius. Conversely, PAR (whereby change in area is divided by the original area) is proportionally minimised in larger wounds. In a retrospective review of 49 VLUs,[11] a strong positive correlation was found between initial ulcer size and healing rate based on area reduction, but no correlation between initial ulcer size and healing rate based on linear advance of the wound margin. This observation of a correlation between ulcer size and measured healing rate (as seen using area reduction as a metric) is problematic for two reasons. The first, and more obvious, is that the healing of large wounds will dominate the results relative to small wounds. The second is that, as wounds generally become smaller over the course of a study, early healing rate will be exaggerated relative to later healing rate, making longitudinal comparisons difficult.

In order to spot a discontinuity (ie, an effect on the healing trajectory) following an intervention, it is of great benefit to have a trajectory that is approximately linear. The advance of the wound margin advance (WMA) towards the centre of the wound (perpendicular to the wound margin) may be calculated for wounds of shapes other than the idealised circular wounds shown in Figure 1. Change in wound area divided by wound perimeter has been proposed as a metric [12] which is directly proportional to the perpendicular advance of the wound margin. This has been shown not only to be equally representative of large and small wounds,[11] but to follow a linear trajectory over time in diabetic foot ulcers, allowing a prediction of healing time by extrapolation after only 4 or 5 weekly measurements. These concur with findings that contraction and epithelialization occurred in a linear fashion perpendicular to the wound edge,[13] and that cell fronts move at constant speed.[14]

WMA has been found to be a powerful predictor of healing[15] and it has been stated that linear healing per unit time should be preferred to measurements of percentage change in wound area to quantify wound healing in clinical trials.[11] Healing rate over 4 weeks has been
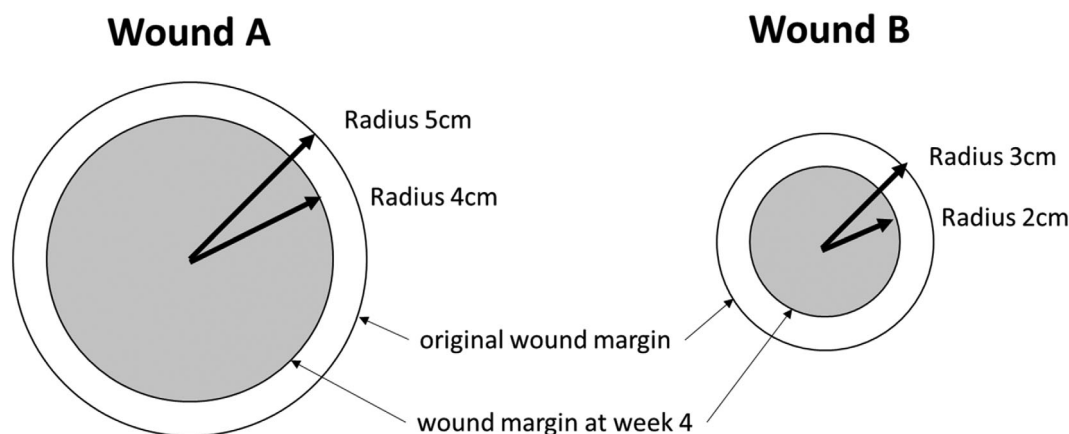
Wound A

Wound B

FIGURE 1 Healing trajectory of two theoretical wounds A and B

TABLE 1 Healing of wounds A and B measured by gross area reduction (GAR)

	Wound A	Wound B
Initial area	$A_1 = \pi r^2 = 79\ cm^2$	$A_1 = \pi r^2 = 28\ cm^2$
Final area	$A_2 = \pi r^2 = 50\ cm^2$	$A_2 = \pi r^2 = 13\ cm^2$
Gross area reduction	$A_1 - A_2 = $ **29 cm²**	$A_1 - A_2 = $ **15 cm²**
Which is healing faster?	✓	

The bold values are the outputs of the calculation (i.e. the results of interest), whereas the other values are inputs.

TABLE 2 Healing of wounds A and B measured by % area reduction (PAR)

	Wound A	Wound B
Initial area	$A_1 = \pi r^2 = 79\ cm^2$	$A_1 = \pi r^2 = 28\ cm^2$
Final area	$A_2 = \pi r^2 = 50\ cm^2$	$A_2 = \pi r^2 = 13\ cm^2$
% Area reduction	$100 \times (A_1 - A_2)/A_1 = $ **37%**	$100 \times (A_1 - A_2)/A_1 = $ **53%**
Which is healing faster?		✓

The bold values are the outputs of the calculation (i.e. the results of interest), whereas the other values are inputs.

TABLE 3 Healing of wounds A and B measured by centimetre advance of the wound margin

	Wound A	Wound B
Initial radius	5 cm	3 cm
Final radius	4 cm	2 cm
Advance of wound margin	$R_1 - R_2 = 1\ cm$	$R_1 - R_2 = 1\ cm$
Which is healing faster?	**equal**	**equal**

The bold values are the outputs of the calculation (i.e. the results of interest), whereas the other values are inputs.

used as an outcome for cost-effectiveness in DFU,[16] and has been used as the primary outcome in RCTs for comparing interventions.[17]

2 | AIMS

The purpose of this study is to determine whether the healing of VLUs receiving multilayer, multicomponent compression bandaging follows a linear trajectory over a 4-week period, as measured by gross area healed, percentage area healed, and advance of the wound margin.

3 | METHODS

Forty VLU patients (24M, 16F) were followed weekly for 4 weeks, with a total of 5 measurements per patient. All patients received standard of care continuously throughout the study, consisting of multilayer, multicomponent compression therapy. Compression systems used were deemed appropriate by the clinical team treating patients in this study.

Patients included were aged ≥18 years and able to provide written informed consent, with chronic VLU between 3 and 39 cm², present for between 6 weeks and 5 years, determined to be due to underlying venous disease, with ankle-brachial pressure index of 0.75 to 1.24, and no active wound infection for a minimum of 48 hours, and no systemic antimicrobial treatment for a minimum of 7 days prior to enrolment.

Patients were excluded if they had known allergy to any of the protocol-stipulated treatments, non-tolerance of multilayer, multicomponent compression therapy intended for the treatment of VLU, history of significant haematological disorders (eg, sickle cell disease), history of deep vein thrombosis within 6 months preceding study

entry, history of pyoderma gangrenosum or other inflammatory ulceration, pregnancy or breast feeding, use of investigational drug or device within 4 weeks prior to study entry that may interfere with this study, use of any neuro-modulation device, or surgery during 3 months prior to study entry (such as abdominal, gynaecological, hip or knee replacement), and participation in any other clinical study.Each ulcer was digitally photographed weekly using the Aranz SilhouetteStar, a digital camera which is part of the Silhouette system. SilhouetteStar is a portable, non-contact device for imaging and measuring ulcers, which has been shown to be more accurate, reproducible, and reliable than manual measurement methods in measuring VLUs.[18,19] All ulcers were photographed pre-debridement. All images were sent to an independent blinded wound expert for delineation of the wound perimeter, from which area and perimeter values were calculated.

Measured wound area was plotted against time at weekly intervals to give a curve for GAR.

PAR was calculated for each wound by dividing measured wound area at each time-point by its initial value at week 0.

According to Vidal's method (below),[12] a value representing WMA corresponding to the rate of advance of the wound edge, was derived by:

$$WMA = d/dt \, (A/P)$$

where A = area, and P = perimeter of the wound.

A value of A/P was calculated at each of the five weekly time-points for each wound. These values were then regressed against time to generate a gradient to represent WMA, as well as a correlation coefficient R^2.

4 | RESULTS

Median wound duration prior to the study was 39 weeks (IQR 14-104 weeks). Median age was 70 (IQR 63-77). Median BMI was 28.2 (IQR 25.4-35.2).

Figure 2 shows GAR relative to the start of the trial. The mean of all 40 patients is plotted against time in days, with error bars representing SE of the means. It can be seen that the trajectory over this 4-week timespan is very close to linear, with a Pearson's R^2 = .95 indicating that the mean values adhere closely to a straight line. Although the SE bars are substantial, this is largely a reflection of the heterogeneity of healing rates within the cohort, rather than non-linearity of healing.

However, by regressing the slope of each individual wound's healing trajectory against initial wound area, GAR correlated positively with initial area (R = .66,
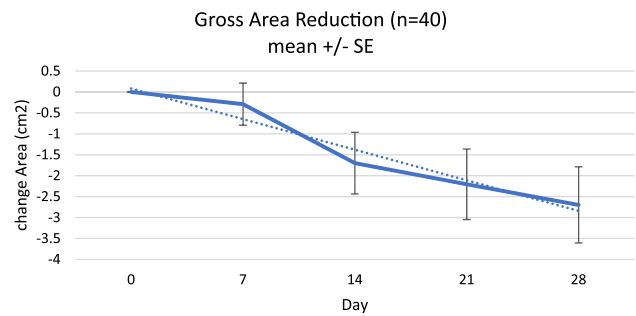

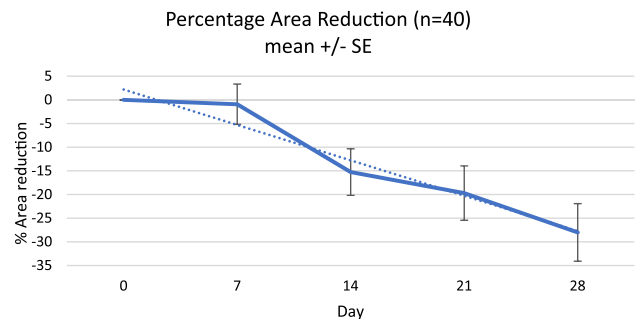**FIGURE 2** Gross area reduction relative to start (n = 40), ±SE

FIGURE 3 Percentage area reduction relative to start (n = 40), ±SE

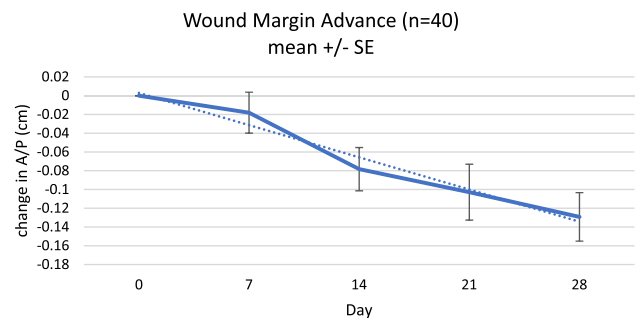**FIGURE 4** Wound margin advance relative to start (n = 40), ±SE

P = .000056), indicating that larger wounds are likely to be over-represented in any measured effect.

Figure 3 shows PAR relative to the start of the trial. The trajectory over this 4-week timespan is also very close to linear, with a Pearson's R^2 = .95 indicating that the mean values also adhere closely to a straight line. In this case, however, the error bars are smaller. Additionally, although PAR showed some weak negative correlation to initial wound area, this was not statistically significant.

Figure 4 shows the mean advance in cm of the wound margin for all 40 wounds, calculated by Vidal's method, at each of the five time-points. WMA follows a linear

trajectory ($R^2 = .97$) correlating even more closely with time than either GAR or PAR. The SEs are not only small, but are relatively uniform at each time-point, giving confidence in the validity of the linear regression. WMA showed no correlation with initial wound area, suggesting that this metric is not biased to large or small wounds.

5 | DISCUSSION

Conducting clinical research into interventions for wound healing is fraught with difficulties. A RCT comparing two separate random cohorts enjoys good external validity in terms of generalisability.[20] However, the internal validity is weak in wound RCTs, where massive heterogeneity in a multiplicity of confounding factors often make comparisons between groups statistically untenable. A Cochrane review[21] of compression for VLU found, out of many thousands of studies, only 48 eligible RCTs, of which only eight had significant stand-alone findings of effectiveness.

The great advantage of the self-controlled trial in terms of internal validity consists in the elimination of noise in the form of inter-group variations, and the elimination of noise resulting from intra-group heterogeneity by the use of pairwise comparisons because each patient acts as their own control, enabling much greater statistical sensitivity.

It has been suggested[22] that self-controlled studies (in which each patient serves as his or her own control) can produce results that are statistically and clinically valid with far fewer patients than would otherwise be required. A consensus document of opinion leaders in wound healing[23] opines that traditional RCTs may not be the most relevant way to measure treatment outcomes, exhorting instead the self-controlled RCT model.

The self-controlled model, however, precludes the use of complete healing as a metric. Clearly, if the wound healed completely with one intervention (or control), it is not possible subsequently to evaluate the effect of healing of another intervention (or control). To reap the sensitivity and validity benefits of a self-controlled study, it is necessary to have an endpoint which can be measured (ideally quantitatively) in the relatively short term, to allow reproduction of the trial with another intervention or control while other factors remain stable. Therefore, it is advantageous to be able to quantify rate of healing in the short term as an endpoint. It is worth noting that rate of healing need not necessarily be regarded as a *surrogate* endpoint for total healing, but perhaps more appropriately an *intermediate* endpoint: it is a benefit per se, and indeed is necessary to achieve healing.

PAR followed over a finite period has been shown to predict complete healing of VLUs.[24] Wounds that ultimately healed were shown to follow a different healing trajectory on average (both by mean and by median) than wounds which ultimately failed to heal. Thus, healing rate in the early weeks could be used to predict complete healing outcome.[15] However, although logistically predictive of healing, the healing trajectory followed by PAR has been reported to be non-linear, and so could not be used easily to predict time to healing. Indeed, it has previously shown a poor correlation to healing time.[25] The non-linear nature of PAR has been variously modelled as approximating exponential[10,26] and as following other trajectories such as a Gompertz function[27,28] and other polynomial fits.[29] So, although shown to be useful as an early classifier of wounds into "healing" or "non-healing" categories, PAR presents non-trivial difficulties in extrapolating a healing trajectory to establish an ultimate time to complete healing. In this study, however, the trajectory of PAR has been found to be close to linear over a 4-week period. This may be explained as follows.

It may be supposed that healing rate in terms of GAR is positively correlated to wound area, as confirmed in this study ($R = .66$, $P = .000056$). As well as showing a higher healing rate for larger wounds in a cohort, this will show a decreasing healing rate over time for individual wounds over the natural history of their healing, as the wound becomes smaller. Thus, over the several months, it may take a wound to heal, a decay curve may be observed as previously reported, as the wound area (a multiplier of measured healing rate) reduces. However, over this relatively short time-span of 4 weeks, the area of the wound generally does not change so appreciably as to cause the healing curve to deviate significantly from a straight line.

PAR differs from GAR only in the fact that each wound's change in area is expressed as a proportion of its initial area. For each wound, this is a constant divisor, and so does not affect the linearity of the healing trajectory. Thus, GAR and PAR both appear equally linear over a 4-week period.

In this study, WMA—corresponding to the advance of the wound margin—followed a remarkably similar trajectory to GAR and PAR. However, WMA adhered even more closely to a linear mean trajectory over a 4-week term. Furthermore, WMA showed no correlation to the initial area of the wound, suggesting no bias towards large or small ulcers in the aggregate.

As with GAR and PAR, it must be noted that this linear trajectory over a 4-week term does not necessarily demonstrate that the trajectory remains linear for its entirety until the wound is totally closed. Rather, linearity in the short term makes it possible to identify any

pronounced discontinuity or change in that trajectory resulting from an intervention being studied.

Neither does these data demonstrate that all wounds heal at the same rate; there is abundant prior observation to the contrary.[30] Chronicity of the wound has been shown to be a risk factor for slow healing (and therefore continued chronicity),[31,32] and it has been suggested that duration of the wound may play a causative role in slow healing, rather than simply being a consequence.[33] Possible mechanisms for this causative link include the obstruction to healing posed by excess inflammation caused by a long-standing wound,[34] and fibroblast senescence.[35] Given that "time is of the essence" in the healing of VLUs, there is a strong argument for aggressive early intervention, and a rapid appraisal of which intervention is or is not working. Whereas time to complete healing does not constitute such a rapid appraisal, measurement of short-term changes to WMA represents a viable prospect for this.

6 | CONCLUSIONS

This study shows that metrics of wound healing—GAR, PAR, and WMA—follow a substantially linear and similar trajectory over a 4-week term. This shows promise for their usefulness in comparing the efficacy of successive interventions in the short term, and speaks to their use as a surrogate for complete healing in clinical trials. The linear nature over this term is important as it enables discontinuities in the mean trajectory (because of an intervention) to be identified. Using a metric of rate of healing in the short term allows self-controlled or crossover studies to be conducted, with huge attendant advantages in statistical power and internal validity compared with conventional unpaired trials.

Of the three metrics investigated, WMA shows the closest adherence to a straight line, and also appears to be independent of initial ulcer size.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Phillips TJ, Dover JS. Leg ulcers. *J Am Acad Dermatol*. 1991;25: 965-987.
2. Hokanson JA, Hayward PG, Carney DH, Phillips LG, Robson MC. A mathematical model for the analysis of experimental wound healing data. *Wounds*. 1991;3:213-220.
3. Falanga V. Care of venous ulcers. *Ostomy Wound Manage*. 1999;45(suppl 1A):33S-43S.
4. Robson MC, Hill DP, Woodske ME, Steed DL. Wound healing trajectories as predictors of effectiveness of therapeutic agents. *Arch Surg*. 2000;135:773-777.
5. Tallman P, Muscare E, Carson P, Eaglstein WH, Falanga V. Initial rate of healing predicts complete healing of venous ulcers. *Arch Dermatol*. 1997;133(10):1231-1234.
6. Polansky M, Van Rijswijk L. Utilizing survival analysis techniques in chronic wound healing studies. *Wounds*. 1994;6: 150-158.
7. Gelfand JM, Hoffstad O, Margolis DJ. Surrogate endpoints for the treatment of venous leg ulcers. *J Invest Dermatol*. 2002;119 (6):1420-1425. https://doi.org/10.1046/j.1523-1747.2002
8. Davis KE, Constantine FC, Macaslan EC, Bills JD, Noble DL, Lavery LA. Validation of a laser-assisted wound measurement device for measuring wound volume. *J Diabetes Sci Technol*. 2013;7(5):1161-1166.
9. Kantor J, Margolis DJ. A multicentre study of percentage change in venous leg ulcer area as a prognostic index of healing at 24 weeks. *Br J Dermatol*. 2000;142(5):960-964.
10. Flanagan M. Wound measurement: can it help us to monitor progression to healing? *J Wound Care*. 2003;12(5):189-194.
11. Gorin DR, Cordts PR, LaMorte WW, Menzoian JO. The influence of wound geometry on the measurement of wound healing rates in clinical trials. *J Vasc Surg*. 1996;23:524-258.
12. Vidal A, Mendieta Zerón H, Giacaman I, et al. A simple mathematical model for wound closure evaluation. *J Am Coll Clin Wound Spec*. 2016;7(1–3):40-49.
13. Snowden JM. Wound closure: an analysis of the relative contributions of contraction and epithelialization. *J Surg Res*. 1984; 37(6):453-463.
14. Maini P, McElwain D, Leavesley D. Traveling waves in a wound healing assay. *App Math Lett*. 2004;17:575-580.
15. Cardinal M, Eisenbud DE, Phillips T, Harding K. Early healing rates and wound area measurements are reliable predictors of later complete wound closure. *Wound Repair Regen*. 2008;16(1):19-22.
16. Waycaster CR, Gilligan AM, Motley TA. Cost-effectiveness of becaplermin gel on diabetic foot ulcer healing changes in wound surface area. *J Am Podiatr Med Assoc*. 2016;106(4): 273-282.
17. Dolibog P, Franek A, Taradaj J, et al. A comparative clinical study on five types of compression therapy in patients with venous leg ulcers. *Int J Med Sci*. 2013;11(1):34-43.
18. Kieser DC, Hammond C. Leading wound care technology: the ARANZ medical silhouette. *Adv Skin Wound Care*. 2011; 24(2):68-70. https://doi.org/10.1097/01.ASW.0000394028. 64777.f7
19. Khashram M, Huggan P, Ikram R, Chambers S, Roake JA, Lewis DR. Effect of TNP on the microbiology of venous leg ulcers: a pilot study. *J Wound Care*. 2009;18(4):164-167.
20. Godwin M, Ruhland L, Casson I, et al. Pragmatic controlled clinical trials in primary care: the struggle between external and internal validity. *BMC Med Res Methodol*. 2003;3(1):28. https://doi.org/10.1186/1471-2288-3-28
21. O'Meara S, Cullum N, Nelson EA, Dumville JC. Compression for venous leg ulcers. *Cochrane Database Syst Rev*. 2012;11(11): CD000265.
22. Louis TA, Lavori PW, Bailar JC, Polansky M. Crossover and selfcontrolled designs in clinical research. *New Engl J Med*. 1984;310(1):24-31.

23. AdvaMed Wound Healing and Tissue Regeneration Sector. Guiding Principles for Clinical Research in Chronic Wound Healing: A Consensus Document. July, 2010.

24. Steed DL, Hill DP, Woodske ME, Payne WG, Robson MC. Wound-healing trajectories as outcome measures of venous stasis ulcer treatment. *Int Wound J.* 2006;3:40-47.

25. Margolis DJ, Gross EA, Wood CR, Lazarus GS. Planimetric rate of healing in venous ulcers of the leg treated with pressure bandage and hydrocolloid dressing. *J Am Dermatol.* 1993;28:418-421.

26. Cardinal M, Phillips T, Eisenbud DE, Harding K, Mansbridge J, Armstrong DG. Nonlinear modeling of venous leg ulcer healing rates. *BMC Dermatol.* 2009;9:2.

27. Wallenstein S, Brem H. Statistical analysis of wound-healing rates for pressure ulcers. *Am J Surg.* 2004;188(1A Suppl):73-78.

28. Renner R, Teuwen I, Gebhardt C, Simon JC. Mathematical model for wound healing following autologous keratinocyte transplantation. *Int Wound J.* 2008;5:445-452.

29. Cukjati D, Rebersek S, Karba R, Miklavcic D. Modelling of chronic wound healing dynamics. *Med Biol Eng Comput.* 2000; 38(3):339-347.

30. Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. The accuracy of venous leg ulcer prognostic models in a wound care system. *Wound Repair Regen.* 2004;12:163-168.

31. Moffatt CJ, Franks PJ, Oldroyd M, et al. Community clinics for leg ulcers and impact on healing. *BMJ.* 1992;305:1389-1392.

32. Franks PJ, Moffatt CJ, Connolly M, et al. Factors associated with healing leg ulceration with high compression. *Age Ageing.* 1995;24:407-410.

33. Bosanquet DC, Harding KG. Wound duration and healing rates: cause or effect? *Wound Repair Regen.* 2014;22(2):143-150. https://doi.org/10.1111/wrr.12149

34. Bello YM, Phillips TJ. Recent advances in wound healing. *JAMA.* 2000;283:716-718.

35. Harding KG, Moore K, Phillips TJ. Wound chronicity and fibroblast senescence—implications for treatment. *Int Wound J.* 2005;2:364-368.